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A SIMPLE METHOD FOR REANIMATING ICE-COLD RATS AND MICE

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Until recently it was supposed that adult non-hibernating mammals succumbed to hypothermia when the deep body temperature fell below about 15° C and that cardiac and respiratory arrest were fatal to them. Then Andjus (1951) reported from Belgrade that he had resuscitated rats with colonic temperatures between 0° and +2° C, and after the heart and breathing had been at a standstill for 40–60 min. Work at the National Institute for Medical Research confirmed and extended Andjus's original findings. Apparatus and techniques were devised which permitted complete revival and long-term survival of 75–100% of adult rats after periods of suspended animation at body temperatures close to zero (Andjus, 1955; Andjus & Lovelock, 1955; Andjus & Smith, 1955; Goldzveig & Smith, 1956). In all the methods so far used, of which microwave diathermy proved the most effective, the cardiac area of the chest was heated preferentially and artificial respiration was administered to the rats. The underlying principle was to restore heart beats and circulation before rewarming the whole body, in order to keep oxygen demands at a low level until supplies were readily available to the tissues.

The same principle was at first applied to resuscitation of hamsters from body temperatures below 0° C. When the cardiac area was heated locally in animals which had frozen for longer than 15 min, complete revival was not effected. When, on the other hand, the whole body was heated by means of conventional short-wave diathermy, hamsters frozen for 16–38 min recovered fully (Smith, Lovelock & Parkes, 1954). It was subsequently found that hamsters progressively frozen for as long as 50 min could be resuscitated not only by this means, but also by gently rewarming the whole body under a 100 W bench lamp (Smith, 1956). It was clear, therefore, that local heating of the heart was neither necessary nor desirable in the partially frozen hamster with arrested respiration and circulation. The question then arose whether

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local cardiac heating was as essential as we had hitherto supposed for resuscitating rats and other non-hibernating mammals from a state of severe hypothermia and suspended animation. Experiments have now been carried out to investigate this problem.

MATERIAL AND METHODS

Animals. Hooded rats ranging in weight from 59 to 190 g were used. The mice used weighed between 22 and 38·5 g and belonged to two different albino strains. One strain, which has been bred at Mill Hill for many years, carries at least three strains of virus and is also susceptible to bacterial hepatitis. The other strain has been maintained at Mill Hill since 1953. It is descended from a few animals originally obtained from the State Serum Institute in Copenhagen, and is housed separately and fed on autoclaved food and water until the time of experimentation. These mice are free from the T.O. strain of encephalomyelitis and have proved particularly hardy. They have been used for a variety of biological studies.

Apparatus and techniques for cooling. The rats were cooled by the method previously described (Andjus & Smith, 1955). The mice were enclosed in Kilner jars (485 ml. capacity) for 58–77 min at −1 to −2° C. They were then cooled in melting ice in the same way as were the rats.

Rewarming by microwave diathermy. The apparatus and technique already described (Andjus & Lovelock, 1955; Perkins, 1955) were used for the rats and were modified slightly for mice. The energy output of the magnetron was decreased to rewarm the mouse heart. This was done by reducing the voltage supply so that the output ammeter read 0·1 A instead of 0·175 A. The power was thus reduced from approximately 150 W to approximately 40 W. A grid extension with a smaller aperture (10 × 0·5 mm) was used. The air output of the automatic apparatus for artificial respiration was decreased. The colonic temperature of mice was measured by specially made small thermometers previously used for hamsters. When the mice had resumed breathing they were not immersed in warm water, but were transferred direct to an incubator at 32–34° C.

Rewarming by illumination from bench lamps. The rats were placed on a wire grid (15 × 9 in., 1cm mesh). The ears, hind-paws and tail lay on strips of adhesive tape and the fore- and hind-paws were also covered with adhesive tape. Bench lamps with shades on the outer side were arranged above and below the animals, as described below. The aim was to raise the colonic temperature by 1° C per min. Unless otherwise stated, the lamps were moved further away if the colonic temperature rose more than 2° C in any minute or if thermometers close to the animals registered above 45° C. Artificial respiration was given until natural breathing was re-established.

The mice were placed on a Perspex support and illuminated by a single shaded bench lamp. An air gap of approximately 3 cm was left between the bulb and the ventral surface of the body. The gap was increased if a thermometer close to the animal registered above 38° C. The rate of increase in colonic temperature was maintained at 1° C per min by altering the position of the lamp.

RESULTS

Resuscitation by means of microwave diathermy. One hundred and four rats weighing 150–190 g were cooled until the colonic temperature had been below 15° C for 1 hr and had reached between +0·1 and +1·2° C. Thirteen of these animals showed no signs of reanimation after rewarming the cardiac area and administration of artificial respiration. Eight others revived partially but died during immersion in water at +38° C. Four rats which had apparently recovered completely died within 24 hr, and another animal died on the 3rd day. There were seventy-eight long-term survivors from this group (Table 1).

Resuscitation by illuminating the whole body. Twelve rats weighing 150–190 g were similarly cooled but were rewarmed by means of three 100 W bench lamps, two of which were arranged as close as possible to the ventral surface and the third beneath the dorsal surface of the body. Artificial respiration was administered. Thermometers close to the rats registered 60° C within 10 min. Three of these animals resumed breathing but died either during immersion in the warm bath or shortly afterwards. The appearance of the skin suggested that the animals had been overheated.

Twelve rats in the same range of body weight were then cooled as before. The two 100 W bench lamps were arranged over the ventral surface of the body, leaving an air gap 5–8 cm in depth. A third 60 W lamp was arranged at the same distance from the dorsal surface. Artificial respiration was administered. After 10 min the air temperature close to the body reached 45° C. Within 14–23 min all these animals resumed natural breathing at colonic temperatures varying from 19 to 21·5° C. Thereafter they recovered completely and have so far survived periods varying from 3 to 15 weeks. (Table 1).

The exact position of the lamps was obviously important. Other lamps were then used to see whether the amount of illumination was also critical. Six rats (150–190 g) were rewarmed by means of one 40 W and two 100 W bench lamps arranged above and below the body. Four of these animals recovered completely and lived for many weeks. The other two failed to revive.

Further reduction in the amount of illumination was not advantageous for rats of 150–190 g. Nine animals in this weight range were given artificial respiration and illuminated from above and below by two lamps, each of 100 W. Seven of these animals appeared to recover completely, but there were three deaths within 24 hr and only four survivors. Illumination by means of one 100 W lamp and one 60 W lamp effected complete resuscitation of one rat, but failed to reanimate another. Twelve smaller rats varying in weight from 59 to 149 g were, on the other hand, successfully resuscitated by combinations of 100 and 60 W, or 100 and 40 W bench lamps. Eleven of these animals are in good health at the time of writing many weeks after reanimation. The twelfth rat was accidentally killed after 16 days (Table 1).

Comparison of reanimation by heating the heart locally and by warming the whole body. Most of the rats which were resuscitated by heating the heart locally resumed natural breathing 11–15 min after the start of treatment at a time when the colonic temperature was between 10 and 15° C. A few animals breathed after as little as 6 or 8 min when the colonic temperature was 8–10° C. Revival was slightly quicker than had been observed by Andjus & Lovelock (1955). Animals rewarmed by illuminating the whole body took somewhat longer to recover. They started to breathe spontaneously 14–23 min after resuscitation was begun when the colonic temperature was between 19 and

24° C. After respiration had become regular it was difficult to detect any difference between rats resuscitated by the two methods.

Special features of cooling mice. The mice cooled more rapidly than the rats, both in closed vessels and when surrounded with melting ice. They became narcotized by the combined effects of hypoxia, hypercapnia and the cold environment after enclosure for approximately 1 hr. At this stage they were breathing deeply and had active corneal reflexes. The colonic temperature was between 13 and 16·5° C. After immersion in icy water the colonic temperature fell very rapidly so that within 5 min it was usually below +5° C, and in a few animals was as low as +1·2° C. The most gradual, the steepest, and the average cooling curves are shown in Fig. 1.

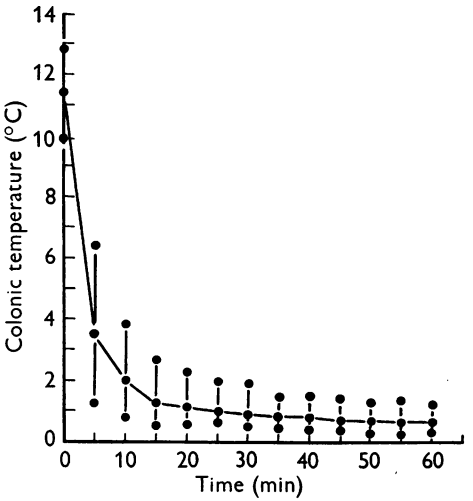

Fig. 1. The fall in colonic temperature of mice immersed in ice-cold water. The highest, lowest and average colonic temperatures recorded at 5 min intervals from fifty mice are shown.

The mice ceased to breathe when the colonic temperature was between +4·5 and +5° C. Electrocardiograms showed that the heart stopped beating when the colonic temperature was between +2·5 and +3·8° C. The mice were kept surrounded by ice until respiration had been arrested for 60 min and until the heart had been at a standstill for approximately 55 min.

Resuscitation by means of microwave diathermy. Eleven albino mice weighing 22–32 g, which had been cooled to body temperatures between +0·2 and +1·1° C, were given artificial respiration while the heart was rewarmed locally. Of the ten animals which revived completely, one died overnight and nine survived for periods varying up to 100 days (Table 2).

Resuscitation by illuminating the whole body. Thirty-three hepatitis-sensitive mice of the virus-carrying strain were cooled in the same way and then given

TABLE 1. Results of reanimating ice-cold rats by local heat on the heart and by illumination of the whole body

Range of body weight (g)	No.	Method of reanimation	No. completely revived	No. partially revived	No signs of revival	Incidence of deaths				Survived 21–100 days or more
						Within 6 hr	6–24 hr	1–7 days	7–20 days	
		Local heat								
150–190	104	Magnetron	83	8	13	—	3	2	—	78
		General illumination								
	12	3 × 100 W	0	3	9	—	—	—	—	0
	12	1 × 60 W 2 × 100 W	12	0	0	—	—	—	—	12
	6	1 × 40 W 2 × 100 W	4	0	2	—	—	—	—	4
	9	2 × 100 W	7	0	2	1	2	—	—	4
	2	1 × 60 W 1 × 100 W	2	—	—	1	—	—	—	1
100–149	9	1 × 40 W 1 × 60 W or 1 × 100 W	9	—	—	—	—	—	—	9
59–99	3	1 × 40 W 1 × 100 W	3	—	—	—	—	—	1	2

TABLE 2. Results of reanimating ice-cold mice by local heat on the heart and by illumination of the whole body

Strain	Range of body weight (g)	No. of animals	Method of reanimation	No. completely revived	No signs of revival	Incidence of deaths				Survived 21–100 days
						Within 6 hr	6–24 hr	1–7 days	7–20 days	
			Local heat							
Mill Hill hepatitis-sensitive	22–32	11	Magnetron	10	1	—	1	—	1	8
			General illumination							
Mill Hill hepatitis-sensitive	23–38·5	33	60 W	33	—	—	1	3	6	23
Copenhagen virus resistant	23–34	21	60 W	21	—	1	1	—	—	19

artificial respiration under a 60 W bench lamp. During the first 3 weeks after resuscitation there were, however, ten deaths. Post-mortem examination showed that these mice had a fulminating hepatitis. The remaining twenty-three mice survived in good health for many weeks (Table 2).

Twenty-one mice of the Danish strain were then cooled and rewarmed in the same way. They all recovered completely. Two died within the first 24 hr, but not apparently from hepatitis. There were no delayed deaths and the remaining nineteen mice had survived in good health for over 2 months at the time of writing (Table 2).

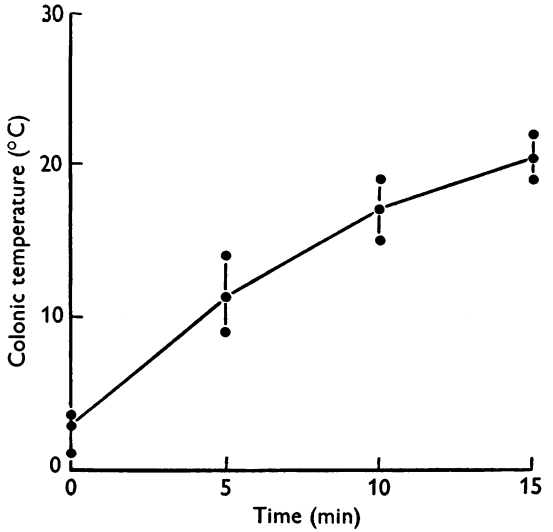

Fig. 2. The rise in colonic temperature of ice-cold mice during administration of artificial respiration and illumination under a 60 W bench lamp. The highest, lowest and average colonic temperatures recorded at 5 min intervals from fifty mice are shown.

Special features of rewarming mice. When the mice were removed from the bath of melting ice they looked like corpses with limp bodies, pallid skin and opaque eyes. During treatment the eyes gradually became red and translucent, and the extremities became pink. The colonic temperature of animals in which the heart was heated locally reached +10° C in approximately 5 min (Fig. 2). They began to breathe naturally after 9–19 min treatment at colonic temperatures between +15 and +23° C. Thirty minutes later they regained normal posture and reflexes in the warm incubator. The colonic temperature of mice treated by illuminating the ventral surface of the body rose rapidly and reached +3° C within 2–3 min, and +10° C within 4–6 min of removal from ice. They resumed spontaneous respiration after 13–17 min at colonic temperatures between 19 and 24° C. There was little difference between the rate of recovery of mice treated by the two methods.

Regular breathing was resumed no sooner by mice than by rats. Subsequently, however, the mice recovered muscle tone, co-ordination and normal activity more rapidly than did the rats. They lost 1–4 g in weight during the first 2 days after resuscitation but, with the exception of animals which succumbed to hepatitis, the mice resumed normal growth before the rats.

DISCUSSION

The results described above show without doubt that adult rats and mice in a state of suspended animation can be revived from body temperatures close to 0° C by warming the whole body instead of by heating the heart locally. The fact that a magnetron microwave generator is not essential and that no other complex apparatus is needed means that these experiments could be repeated in any ordinary laboratory.

Ice-cold rats can seldom be resuscitated by a combination of artificial respiration, and either incubation in warm air or immersion in warm water. It was therefore surprising that gentle warming by illumination with a simple arrangement of bench lamps effected the resuscitation and long-term survival of thirty adult and two immature rats. It is felt that more experience is needed before optimum conditions can be laid down. Nevertheless, from a theoretical point of view, these results are of great importance and suggest that the danger of tissue anoxia has been exaggerated.

Resuscitation of ice-cold mice by artificial respiration and illumination by a single bench lamp was particularly easy and preferable to the use of the magnetron. It was interesting that acute attacks of hepatitis were precipitated in a high proportion of susceptible mice shortly after exposure to and recovery from severe hypothermia. This result indicated that resistance to intercurrent infection had been reduced. The mice of a hardier strain, however, showed no apparent after-effects.

SUMMARY

1. Adult rats and mice were cooled until their colonic temperatures reached between +1·2 and 0° C, and until heart beats and breathing had been arrested for 50–60 min.

2. They were treated by administration of artificial respiration and by warming. In some animals heat was focused on the heart by means of microwaves generated by a magnetron. Other animals were rewarmed by illuminating the whole body with bench lamps.

3. A high proportion of animals were resuscitated by both methods and survived for long periods thereafter. The simple apparatus and technique for rewarming the whole body had obvious advantages.

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